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(54)【発明の名称】　糖尿病予備群の検査方法

(57)【要約】
【課題】　患者から分離した試料を用いて、非糖尿病患者と、糖尿病予備群を含む糖尿病患者とを簡便かつ正確に分別する方法。
【解決手段】　試料を前処理することなくミオイノシトールを分解する酵素で処理し、試料中に含まれるミオイノシトールを分解し、その分解物を測定し、試料中のミオイノシトール含量を定量する。その定量値によって、正常型、糖尿病予備群（境界型、耐糖能異常、空腹時血糖異常上昇、インスリン抵抗性）及び糖尿病を分別する。酵素としては、デヒドロゲナーゼ、キナーゼ、オキシダーゼ等が用いられる。

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TITLE: METHOD FOR EXAMINING PRELIMINARY GROUP OF DIABETES MELLITUS

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ABSTRACT:

PROBLEM TO BE SOLVED: To provide a method for simply and accurately discriminating non- diabetes mellitus group patients and diabetes mellitus group patients including diabetes mellitus preliminary group by using samples separated from patients.

SOLUTION: A sample is treated with an enzyme decomposing myoinositol without carrying out pretreatment, and myoinositol contained in the sample is decomposed and the decomposed material is measured to determine myoinositol content in the sample. A normal type, diabetes mellitus preliminary group (boundary type, impaired glucose tolerance, impaired fasting glysemia, insulin resistant type) and diabetes mellitus are discriminated by the determined value. Dehydrogenase, kinase, oxykinase, etc., is used as the enzyme.

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
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CLAIMS

[Claim(s)]
[Claim 1] The inspection approach which processes the myo inositol contained in it with the enzyme which acts on a myo inositol, measures the product, carries out the quantum of the amount of the myo inositol contained in a sample, and is characterized by judging beyond a characteristic value to be a diabetes-mellitus reserve group or diabetes mellitus, without pretreating a sample.
[Claim 2] The approach according to claim 1 characterized by distinguishing a reserve group by removing diabetes mellitus from the group judged to be a diabetes-mellitus reserve group or diabetes mellitus.
[Claim 3] The approach according to claim 1 to 2 characterized by a sample being a sample obtained by after a sugar load or after a meal.
[Claim 4] The characteristic value whose sample is urine Approach according to claim 1 to 3 characterized by being 19microg/mg and KUREANICHIN after 75g glucose tolerance test.
[Claim 5] The characteristic value whose sample is urine Approach according to claim 1 to 3 characterized by being 16microg/mg and KUREANICHIN after 75g glucose tolerance test.
[Claim 6] The characteristic value whose sample is urine Approach according to claim 1 to 3 characterized by being 13microg/mg and KUREANICHIN after 75g glucose tolerance test.
[Claim 7] The inspection approach characterized by judging extent of abnormal glucose tolerance by carrying out the quantum of the amount of the myo inositol contained in the sample.
[Claim 8] The approach according to claim 1 to 7 that the enzyme which acts on a myo inositol is characterized by being a dehydrogenase, a kinase, or an oxidase.
[Claim 9] A dehydrogenase is Klebsiella pneumoniae. (Klebsiella pneumoniae) TK24 (FERM BP-6506), Bacillus sp. (Bacillus sp.) No.3 (FERM BP-5881) Or Flavobacterium ESUPI (Flavobacterium sp.) The inspection approach according to claim 8 which is the dehydrogenase of the 671 (FERB BP-7323) origins.
[Claim 10] The approach according to claim 1 to 9 the approach of measuring a myo inositol is the enzyme cycling method.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the inspection approach of extent of a normal type, a diabetes-mellitus reserve group, and abnormal glucose tolerance. Setting to this invention, a normal type is 75g. When an oral glucose tolerance test is performed, the group which are less than 110 mg/dl of fasting blood sugar levels and less than 140 mg/dl of after [a load] 2-hour blood sugar is pointed out. Moreover, a diabetes-mellitus reserve group is a boundary mold, abnormal glucose tolerance (impaired glucose tolerance, IGT), and the abnormality rise in a fasting blood sugar level (impaired fasting glysemia, IFG). Or what is insulin resistance is said. Furthermore, or it sorts out by the decision criterion which WHO presented by the glucose tolerance test in 1998, distinguish from diabetes mellitus with the value of HbA1c based on the Japanese old ** method. In addition, judge with blood sugar markers, such as fructosamine, the Glico albumin, and a 1.5 anhydro KISHIGURUSHI toll. It sorts out by the decision criterion which WHO presented with the fasting blood sugar level in 1998, and as long as it can perform a diabetic judgment also except this, it may be judged using which approach. according to this invention -- a clinical laboratory test -- setting -- usefulness -- extent of a normal type, a diabetes-mellitus reserve group, and abnormal glucose tolerance can be inspected simple and cheaply.
[0002]
[Description of the Prior Art] Inspection of a diabetes-mellitus reserve group, division, a boundary mold, IFG, and IGT Or inspection of insulin resistance is very important from a viewpoint of prevention of diabetes mellitus and a lifestyle-related disease as well as decision of medication of insulin resistance discharge medicine, and the check of the effectiveness. Moreover, diabetes-mellitus reserve groups are a diabetic and an EQC (80 Yano.K, Kagan A, et.al.Am JMed 721:71- 1996) as a risk of ischemic heart disease, the break in equivalent to a diabetes-mellitus group is also called need, and it is thought like detection of a diabetes-mellitus group that detection of a diabetes-mellitus reserve group is important.
[0003] a normal type, a boundary mold, IFG, and IGT and a diagnosis of a diabetes-mellitus mold -- 75g it diagnoses by the glucose tolerance test -- having -- insulin resistance -- the insulin reaction in blood at the time of a glucose tolerance test, an insulin intravenous-injection test, and Steady state plasma glucose -- law and Hyperinsulinemic euglycemic glucose clamp -- although it could measure by the trial of law, a minimal model method, etc., any approach had a great effort and time amount, and the fault for which complicated actuation is needed. For this reason, development of the simple diagnostic approach and the predicting method is desired.
[0004] On the other hand, it is thought that the quantum of the quantum of the inositol in a living body especially the Cairo inositol, and a myo inositol is useful to prediction of a diabetes-mellitus condition. It is the following about the example which measured change of the amount of inositols in the urine in diabetes mellitus and non-diabetes mellitus, and blood. (a) - (f) It is shown.
(a) The Cairo inositol concentration in urine falls of diabetes mellitus [Larner J.et al., New Eng.J.Med., 323,373-378 (1990)].
(b) The Cairo inositol concentration in urine rises of diabetes mellitus, and, as for the Cairo inositol

concentration in blood, diabetes mellitus does not change, either [Richard E.Ostland, Jr, Proc.Natl.Acad.Sci.USA, Vol.90 9988(1993)-9992].
(c) The myo inositol concentration in urine rises of diabetes mellitus [Larner J.et al., New Eng.J.Med., 323,373-378 (1990)].
(d) The myo inositol concentration in blood does not change of diabetes mellitus [Richard E.Ostland, Jr, Proc.Natl.Acad.Sci.USA, Vol.90 9988(1993)-9992].
(e) The myo inositol concentration in blood is [Nakajima ** [which goes up of diabetes mellitus], Japanese clinical, special number number, blood, urine chemistry inspection and immunologic test, and 4th edition first volume (1995)738-741].
(f) The myo inositol concentration in blood rises of diabetes mellitus [Roswitha Dolhofer, O.H.Wieland., J.Clin.Chem.Clin.Biochem., and 25,733-736 (1987)].
[0005] Above (a) - (f) The normal type which measures change of the inositol in diabetes mellitus and non-diabetes mellitus altogether, and is classified according to measurement of an inositol with non-diabetes mellitus from this result, a boundary mold or a normal type, and IFG And IGT That it can distinguish cannot predict. Because, even if the quantum value of a certain marker rises by the diabetic, they are a boundary mold, IGT, and IFG. It is because the said diabetes-mellitus reserve group does not necessarily go up, either. For example, at the glycosylated hemoglobin which is a diabetic marker, even if a quantum value rises intentionally by the diabetic, it is shown clearly in the boundary mold that it does not go up. The above since it is the same (a) - (f) A result to a normal type, a boundary mold or a normal type, and IFG And IGT That it can distinguish cannot predict. further -- (a) - (e) although all are measured using GC-MASS -- (a) And (b) Or (d) And (e) like -- since the result changes with operating personnels, a problem is in the repeatability and dependability of data.
[0006] It is change of the amount of inositols in the urine in a boundary mold, and insulin resistance and a normal type, and blood GC-MASS It is the following about the example used and measured. (g) - (h) ******.
(g) Decreasing, if the amount of Cairo inositols in urine becomes a boundary mold and insulin resistance compared with a normal type, the amount of myo inositols in urine does not change in a normal type and a boundary mold. [Susumu Suzuki, Diabetes Care, Vol.17, No12 (1994) 1465-1468].
(h) In a prediabetic state, the Cairo inositol in urine goes up intentionally compared with a normal type after sugar administration (Japanese-Patent-Application-No. 11-106979 number). . Such (g) (h) Above (a) - (e) Although similarly measured using GC-MASS, since the result changes with operating personnels, a problem is in the repeatability and dependability of data. according to examination of this invention persons, a healthy person's inositol concentration in the living body is included 1-50microM the inositol concentration field where extent is low -- GC-MASS the place which performed the recovery test using law -- very low recovery -- becoming -- GC-MASS law -- it was checked that a problem is in the dependability of data.
[0007] The approach of carrying out the quantum of the inositol is above mentioned GC-MASS. Immunoassay (JP,8-21835,A), enzymatic process [Roswitha Dolhofer, O.H.Wieland., J.Clin.Chem.Clin.Biochem., and 25,733-736 (1987)], etc. using an antibody specific to the report [Toshimitsu Niwa, J.Chromatography, 227 (1983), 25-39] which carried out the quantum, and the Cairo inositol are known.
[0008] Pretreatment is required, and it is reproducible, moreover, since actuation is complicated, said GC-MASS method has a problem in dependability, and multi-specimen processing is also still more difficult for it. While said immunochemistry-approach is high sensitivity, it has a problem in respect of the effect of the low-molecular matter which exists so much in repeatability and a living body, the specimen throughput per unit time amount, cost, etc. Moreover, said enzymatic process needs pretreatment of a complicated sample, and processing of many specimens is difficult for it.
[0009] Then, this invention persons have a high precision and high sensitivity enzymatic process which does not need pretreatment for the purpose of offering the quantum approach of a simple and cheap inositol has been developed (about JP,6-61278,B and the Cairo inositol, it is [myo inositol] WO 98-42863). and pretreatment -- unnecessary enzymatic process became possible and acquisition of data

reliable about an inositol for the first time was attained.
[0010]
[Problem(s) to be Solved by the Invention] The purpose of this invention is to offer the inspection approach of the diabetes mellitus which judges whether it corresponds to diabetes-mellitus reserve groups, such as a boundary mold on a boundary with whether it is the normal type with which the candidate has not done the illness of the diabetes mellitus, and diabetes mellitus, IGT, IFG, or insulin resistance, using samples, such as a blood serum, plasma, and urine, with simple and sufficient repeatability, especially a diabetes-mellitus reserve group.
[0011]
[Means for Solving the Problem] In order to attain the above-mentioned purpose, it is related with the approach of sorting out in a normal type and an abnormal mold using the measuring method of the myo inositol by the simple enzyme which does not need pretreatment. furthermore, this invention -- a diabetes-mellitus reserve group, i.e., a boundary mold, IGT, and IFG it is -- a sample including a candidate is measured and it is related with the quantum value which can recognize a normal type, a diabetes-mellitus reserve group, or diabetes mellitus clearly. Furthermore, diabetes mellitus is a glucose tolerance test. HbAlc based on the Japanese old ** method or it sorts out by the decision criterion which WHO presented in 1998 Distinguish with a value. In addition, fructosamine, the Glico albumin, and 1.5 Judge with blood sugar markers, such as an anhydro KISHIGURUSHI toll. He is WHO by the fasting blood sugar level. It sorts out by the decision criterion shown in 1998, and as long as it can perform a diabetic judgment also except this, it may be judged using which approach.
[0012] Then, this invention persons are simple approaches not to need pretreatment using the enzyme which acts on a myo inositol as a result of examination wholeheartedly. And unlike that the myo inositol in a sample can be measured with sufficient repeatability, and the knowledge of the former also still more unexpectedly, as compared with it of the candidate whose myo inositol quantum value of the candidate who is a diabetes-mellitus reserve group is a normal type, it goes up intentionally. Namely, the thing it can judge that is a diabetes-mellitus reserve group or diabetes mellitus by the high probability when it is beyond the value of a characteristic value, By measuring the measured value of diabetes-mellitus markers (for example, a fasting blood sugar level, glycosylated hemoglobin, saccharification albumin etc.) furthermore, and adding whether it is beyond the value of the characteristic value of a normal type It found out that the judgment of a diabetes-mellitus reserve group, diabetes mellitus, or insulin resistance could be performed in a still higher probability. What is necessary is just to inspect and except diabetes mellitus by the well-known approach.
[0013] That is, this invention is made in order to attain such a purpose, the quantum of the inositol in a sample is carried out without pretreatment using an enzyme, and the quantum value is the inspection approach of judging a diabetes-mellitus reserve group or diabetes mellitus for the case of being intentionally high, compared with a characteristic value. It is related with the inspection approach of judging a diabetes-mellitus reserve group, by furthermore removing diabetes mellitus from a diabetes-mellitus reserve group or diabetes mellitus.
[0014] In this case, compared with the characteristic value which the quantum value set up beforehand based on the average of a normal type, and standard deviation as it is high, a high thing is said intentionally. and the case where the sample of a characteristic value is a blood serum or plasma -- 27micromol/L it is -- the case where a sample is urine -- 19microg/mg and a creatinine, or 16microg/mg and a creatinine -- they are 13microg/mg and a creatinine preferably. Moreover, a large-scale trial will be performed from now on, and when the normal type decided clinically is judged, this characteristic value may change. And as for the quantum of an inositol, it is desirable to carry out using the enzyme which acts on an inositol at least. About this average, the average changes, for example with selections of the population, such as a race, sex, and age. As a sample, the blood serum or plasma separated from the body is used. Moreover, the urine obtained by non-invasion as a sample is used.
[0015] Furthermore, this invention carries out the quantum of the myo inositol in a sample using an enzyme, and that quantum value judges that the case of being intentionally high is a diabetes-mellitus reserve group, diabetes mellitus, or insulin resistance compared with the characteristic value of a normal

type, and it relates to the inspection approach of of the diabetes-mellitus reserve group and insulin resistance which become by removing diabetes mellitus from this inside. as such a diabetes-mellitus marker -- a fasting blood sugar level, glycosylated hemoglobin, and saccharification -- albumin, fructosamine, etc. can be illustrated.

[0016] Hereafter, it explains in more detail about this invention and its desirable gestalt. Which sample may be used as long as it is the sample which can judge a normal type, a diabetes-mellitus reserve group, or diabetes mellitus when **** is separated from the body by living body liquid, such as the blood separated from the body, for example, whole blood, plasma, a blood serum and a corpuscle, or urine, etc. mentioning as a sample of this invention and samples other than this also measure a myo inositol. If a test subject's burden is taken into consideration as a desirable sample, the urine obtained by non-invasion will be mentioned. Moreover, although the inspection approach of the diabetes-mellitus reserve group of this invention may collect samples regardless of a sugar load or a meal, since a diabetes-mellitus reserve group can be inspected with the still more sufficient sensibility after a sugar load and to after a meal, after a sugar load and a sample after a meal are desirable. After a sugar load and after a meal point out the glucose tolerance test or meal back, an oral load is desirable as a glucose tolerance test, Glucoses 50-200g are desirable as a burden, and 75g is the most desirable. The time amount which extracts a sample is after a sugar load and 0-6 after a meal. The sample of time amount is desirable and it is 0.5-3. The sample of time amount is the most desirable.

[0017] In order to carry out the quantum of the myo inositol, as long as it is the approach of carrying out the quantum of the myo inositol using an enzyme, you may measure using which approach.

[0018] As long as it is the enzyme which acts on a myo inositol at least as an enzyme which can carry out the quantum of the myo inositol which can be used for this invention, what kind of enzyme may be used. It cannot be overemphasized that enzymes, such as the enzyme which acts on a well-known myo inositol, for example, inositol oxygenase, (E. C.1.13.99.1), an inositol dehydrogenase (E. C.1.1.1.18), an inositol kinase (E. C.2.7.1.64), and inositol methyltransferase (E. C.2.1.1.39), can use it for this invention. Moreover, as a result of this invention persons' screening the enzyme which acts on a myo inositol from a nature widely, it found out producing the dehydrogenase to which various bacteria act on a myo inositol. The example of the dehydrogenase production bacillus which acts on a myo inositol is shown below. However, it does not pass over these to one example, and they do not restrict this invention at all.

[0019] (1) Bacillus (Batillus); -- bacillus Subtilis (subtilis;PCI-219 stock --) IFO-13586 A stock, IAM-1026, NRRL B-3639, SUFAERIKASU (sphaericus;IAM -1286) bacillus Wren -- TASS (lentus) and bacillus - Bacillus cereus (scereus;IFO-3009) Bacillus sp. No.3 (sp.No.3; FERM BP-5881), Bacillus PAMIRUSU (55 pumilus;Appl Microbiol and Bioteck 15(1)52- 1982).

(2) Brevibacterium (Brevibacterium); BUREBI bacterium AMONIAGENESU (ammoniagenes;IAM - 1641), BUREBI bacterium DIBARIKATSUMU (divaricatum;NRRL-2311).

[0020] Pseudomonas (Pseudomonas); (3) Pseudomonas fluorescens (fluorescens), Pseudomonas ESUPI (sp.) and Pseudomonas aeruginosa (aeruginsa;NTCT-10490), Pseudomonas malto FIRIA (maltophilia;IFO-12020), Pseudomonas putida (putida), Pseudomonas MEFITIKA (mephitica), the Pseudomonas BAIE phosphorus key (1337 beijerinckii;Monatshe Chem 1001327- 1969).

[0021] (4) Yeast group (Yeast); Cryptococcus MERIBIOSAMU (Cryptococcus-melibiosum;IGC - 3939).

(5) Xanthomonas (Xanthomonas); xantho MONASU ORIZE (oryzae;IFO12000).

(6) Vibrio (Vibrio); vibrion ESUPI (sp.).

(7) Streptococcus group (Streptococcus); streptococcus DISUGARAKUCHIE (dysgalactiae).

(8) Agrobacterium (Agrobacterium); an Agrobacterium tumefaciens (tumefaciens;ATCC-4720), Agrobacterium RADIOBAKUTA (radiobacter; IFO-13259), Agrobacterium rhizogenes 1215 (rhizogenes1215;FERM BP-6270).

[0022] (9) Micrococcus (Micrococcus); Micrococcus lysodeikticus (lysodeiktcus;IFO -3333), micrococcus RUTEUSU (luteus;IFO -3067), micrococcus guru TAMIKASU (glutamicus;ATCC - 13032).

(10) Enterobacter (Enterobacter); Enterobacter aerogenes (aerogenes).
(11) Corynebacterium (Corynebacterium); Corynebacterium AKUA tee cam (aquaticum;IFO-12154).
[0023] (12) Arthrobacter (Arthrobacter); Arthrobacter pro TOFORUMIA (protophormia).
(13) Genus erwinia (Erwinia); ERIBINIA ESUPI (sp.).
(14) Klebsiella group (Klebsiella); Klebsiella pneumoniae TK24 (pneumoniae; FERM BP-6560).
(15) Neurospora group (Neurospora); Neurospora crassa (717 crassa;Biochim Biophys Acta 136 (3) 1977).
(16) Aerobacter group (Aerobacter); Aerobacter AEROGENESU (806 aerogenes;J.B.C.241 (4) 800- 1966).
(17) Acetomonas (Acetomonas); Aceto MONASU oxydans (oxydans;NCIB -621).
[0024] (18) Acetobacter (Acetobacter); Acetobacter suboxydans (10 suboxydans;Helv Chem acta 50(7) 1801- 1967).
(19) Serratia (Serratia); Serratia marcescens (328 marcescens;Allg Mikrobiol 16(4)327- 1976).
(20) Rhizobium (Rhizobium); rhizobium Tori Foley (1114 trifolii;j.Bacteriol 141 (3) 1109- 1980).
(21) Streptomyces (Streptomyces); streptomyces ROZEO griseus 301 (roseogriseus 301;FERM BP-6269).
(22) Escherichia (Escherichia); Escherichia coli (coli;NCTC -8959).
(23) Flavobacterium (Flavobacterium); Flavobacterium ESUPI (sp.; ATCC-21429, FERM BP-7323) and the Flavobacterium AKUA tile (aquatile;IFO -3772).
(24) Animal origin; mammalian brain origin myo inositol oxidoreductase (1138 BioChemBiophys Res Commun 68(4)1133- 1976) and an animal sperm origin inositol dehydrogenase (1700 Dokl Bolg Aked Nauk 24 (12) 1699- 1971).
[0025] Rather than the Cairo inositol, if it is a dehydrogenase with high singularity, and the enzyme which acts on a myo inositol preferably and does not act on the Cairo inositol substantially, anything can be used for a myo inositol at the purpose which measures only the myo inositol in a sample. As the example, it is Japanese Patent Application No. The enzyme which Klebsiella pneumoniae (Klebsiella pneumoniae) TK24 (it abbreviates to Kp.TK24 below FERMBP-6506;.) and Flavobacterium ESUPI (Flavobacterium sp.) 671 (it abbreviates to F.sp.671 FERM BP-7323 and the following.) given in No. 270948 [ten to] (1998 September 25 application) produce is mentioned.
[0026] In addition, 24 shares of Kp.TK(s), three shares of B.sp.No(es), and F.sp.671 share are deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of the Japan 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken whereabouts, the Ministry of International Trade and Industry, as trust number FERM BP-6506 (trust day September 17, Heisei 10), trust number FERM BP-5881 (trust day March 19, Heisei 9), and trust number FERM BP-7323 (trust day October 12, Heisei 12), respectively.
[0027] K.p. It is a gram-negative short Bacillus, and since 24 shares of TK(s) had that they are the facultatively anaerobic bacteria which do not form a spore, that there is no maneuverability, and the gas generation from a lactose, they were Escherichia coli, from generation of the acid from enzyme production ability, such as catalase production and urease production, and sugar etc., they were identified Klebsiella pneumoniae subESUPI pneumoniae (Klebsiella pneumoniae subsp.pneumoniae), and named the bacteria stock 24 shares of Klebsiella pneumoniae (Klebsiella pneumoniae) TK.
[0028] F. There is [that sp.671 share is a gram-negative Bacillus and it is aerobe,] no maneuverability, It is the Flavobacterium (Flavobacterium) group from enzyme production ability, such as that Pori beta hide ROKISHI butanoic acid does not exist in intracellular organelle, catalase production, oxidase production, and phosphatase production, Moreover, it is identified Flavobacterium ESUPI (Flavobacterium sp.) 671 (FERM BP-7323) from the gas generation from sugar etc., and is Flavobacterium ESUPI (Flavobacterium sp.) 671 about a bacteria stock. It was named the stock.
[0029] B. The sp.No.3 share mycology-property is opened to WO No. 42863 [98 to] official report.
[0030] As a dehydrogenase production bacillus used for this invention, it is 24 shares of above Kp.TK (s), and B.sp.No.3. Although a stock, F.sp.671 share, etc. are mentioned bacteria are common -- as description, since the property on mycology may vary The UV irradiation naturally or usually

performed, radiation irradiation, or a variation inducer, For example, N-methyl-N'-nitro -N - The artificial variant which may vary with the artificial variation means using nitrosoguanidine, ethyl methanesulfonate, etc., of course It belongs to a Klebsiella group or Flavobacterium also including a spontaneous mutation stock. The strain which has the capacity to produce a dehydrogenase with high singularity to a myo inositol, Moreover, the strain which has the capacity to produce the dehydrogenase which belongs to Bacillus and acts on a myo inositol and the Cairo inositol can be altogether used for this invention as a purpose which computes a myo inositol except for the Cairo inositol from inositols. As a purpose which computes a myo inositol except for the Cairo inositol from inositols, the transformation microorganism which introduced into the microorganism the gene which discovers the above-mentioned dehydrogenase can also be used. The same is said of the kinase and oxidase which act on an inositol.

[0031] The conditions generally used to bacterial culture can perform the above-mentioned culture. The carbon source which a microorganism can assimilate as a culture medium, the nitrogen source which may be digested, and the nutrition culture medium which made mineral salt etc. contain if needed further are used. as a carbon source which can be assimilated, a glucose, a fructose, saccharose, an inositol, etc. are independent -- or it is combined and used. as a nitrogen source which may be digested, a peptone, a meat extract, a yeast extract, etc. are independent, for example -- or it is combined and used. In addition, various heavy-metal salts, such as phosphate, magnesium salt, a calcium salt, potassium salt, sodium salt, other iron, and manganese, etc. are used if needed. Moreover, the carbon source well-known in addition to the above which can be assimilated, and the nitrogen source which may be digested can be used.

[0032] Deep part aeration spinner culture is industrially [often / usually performing culture under aerobic conditions, such as shaking culture or aeration spinner culture, /, and] desirable. Culture temperature is usually 20-60 degrees C and B.sp.No.3, although it can change suitably within limits which a production bacillus grows and produce the aforementioned enzyme. Especially with a stock, near 50 degree C is Kp.TK24. As for a stock and especially F.sp.671 share, near 30 degree C is desirable. although culture time amount changes with culture conditions, said enzyme chooses the stage to reach a high potency at its own discretion and is suitable for it -- what is necessary is just to carry out time amount culture Usually, it is about one - two days. Accommodation selection is suitably made so that a desirable result may be obtained according to these medium compositions, the acidity or alkalinity of a culture medium, culture temperature, agitating speed, permeability, etc. Moreover, when there is foaming in liquid culture, defoaming agents, such as silicone oil and vegetable oil, are used suitably.

[0033] Thus, since the obtained dehydrogenase is contained mainly in a fungus body, a harvest is carried out with the means of filtration or centrifugal separation from the obtained culture, and the enzyme content liquid of rough purification is obtained in this fungus body, combining suitably various fungus body processing means, such as enzyme-destructive means, such as mechanical destructive means, such as sonication, French press processing, glass bead processing, and freezing crushing processing, and a lysozyme.

[0034] Purification is performed combining suitably the salting-out settling which adds an ammonium sulfate, a sodium sulfate, etc., a molecule sieve, the chromatography using various kinds of resin, various electrophoresis and ultracentrifugation, the various desalting methods, heating, processing by chemicals, etc. For example, as a chromatography, a cation, an anion-exchange chromatography, gel permeation chromatography, distribution, adsorption chromatography, a normal phase, reversed phase chromatography, a canal chromatography, hydroxyapatite chromatography, affinity chromatography, etc. can be used.

[0035] The preservation after purification can save liquefied or a freeze-drying article on condition that refrigeration, freezing, etc. Moreover, in liquefied preservation, at the time of freeze drying, it is good to add various stabilizing agents in order to maintain the solubility at the time of the dissolution, and the stability after the dissolution in order to save an enzyme at stability. It is mentioned as a stabilizing agent, divalent metal ions, for example, magnesium chlorides etc., such as peptides, such as amino acid, for example, glutamic acid, such as a saccharide, for example, a mannitol, saccharose, and a sorbitol,

and a glycine, or protein, for example, albumin etc., etc. The amount of the stabilizer used 0.1 - 50% is desirable.

[0036] The activity measurement method of the inositol dehydrogenase of 24 shares of refined Kp.TK (s), three shares of B.sp.No(es), and the F.sp. 671-share origin is as follows.

(1) Activity measuring method <reaction mixture presentation> 100mM Tris-buffers (pH8.5) 20mM A myo inositol (U.S. by the sigma company), or Cairo inositol (Wako Pure Chem Japan)
2mM(s) Nicotinamide adenine dinucleotide (NAD) (oriental yeast company make, Japan)
5U/ml Diaphorase (the Asahi Chemical Industry Co., Ltd. make, Japan)
0.025 % nitroblue tetrazolium (NBT; Wako Pure Chem make)
1.5 % Triton (Triton) - X100 (Wako Pure Chem Make, Japan)

[0037] the 1ml of the above-mentioned reaction mixture a small test tube -- putting in -- 37 degrees C -- 5 a part -- between -- incubation -- after -- enzyme liquid 20microl diluted B times It adds, and it stirs and a reaction is started. It is 5 correctly. It is 0.1NHCl 2ml after the reaction between parts. It adds, and it stirs and a reaction is suspended. 550nm The absorbance which can be set is measured and A1 is calculated, and same measurement is performed using reaction mixture excluding the myo inositol or the Cairo inositol from the above-mentioned reaction mixture, and it asks for the absorbance A0. Enzyme activity is computed from the following formula.

[0038]

[Equation 1]

$$U/ml = \frac{(A1-A0)}{18.3} \times \frac{1}{5} \times \frac{3.02}{0.02} \times B$$

[0039] The numeric value in a formula means a degree.

18.3 ; NTB Molar Extinction Coefficient 5 ; Reaction Time 3.02; The Total Reaction Volume 0.02; Enzyme Volume B ; Dilution Scale Factor of Enzyme Liquid [0040] The description of the inositol dehydrogenase of 24 shares of refined Kp.TK(s), three shares of B.sp.No(es), and the F.sp. 671-share origin is as follows.

(2) an enzyme operation -- even if few, generate INOSOSU and a reduction type coenzyme under existence of a myo inositol or the Cairo inositol, and a coenzyme. About the above-mentioned coenzyme, nicotinamide adenine dinucleotides (henceforth, NAD it abbreviates to a kind) (NAD), for example, nicotinamide adenine dinucleotide, acetyl pyridine adenine dinucleotide (acetyl NAD), nicotinamide hypoxanthine dinucleotide (deamino NAD), pyridine aldehyde adenine dinucleotide (aldehyde NAD), and pyridine aldehyde hypoxanthine dinucleotide (aldehyde deamino NAD) are mentioned. The relative activity ratio (the case where NAD is used as a coenzyme is made into 100 %) at the time of using each coenzyme is as being shown in Table 1. Moreover, relative activity measured by changing a coenzyme based on the following approach.

[0041]

[Table 1]

各補酵素を用いた場合の相対活性比

菌株名	K. p. TK24	B. sp. No. 3	F. sp. 671
補酵素	ミオイノシトール	カイロイノシトール	ミオイノシトール
NAD	100 %	100 %	100 %
アセチルNAD	6 %未満	13 %	— %
デアミノNAD	59 %未満	115 %	— %
アルデヒドNAD	4 %未満	11 %	— %
NADP	0 %未満	3 %	8 %
チオNAD	0 %	20 %	29 %
チオNADP	0 %未満	0 %	0 %

[0042]
Relative activity measurement method <Reaction mixture presentation> Buffer solution 100mM Glycine buffer solution (pH10.0)
Substrate 20mM Myo inositol (24 shares of Kp.TK(s), and F.sp.671 stock origin) 10mM Cairo inositol (B. sp.No.3 stock origin)
Coenzyme 2mM (24 shares of Kp.TK(s), F.sp. 671-share origin) 1mM (B. sp.No. three-share origin) (More than thio NADP; Japan by the oriental yeast company, the acetyl NAD, Aldehyde NAD, and more than deamino NAD ; NAD, Thios NAD and NADP, U.S. by the sigma company) .
[0043] The 1ml of the above-mentioned reaction mixture It sets to the spectrophotometer by which the temperature control is carried out to 37 degrees C for a quartz cell. It incubates 5 minutes or more and is about 1.0U/ml. 20microl is added and an enzyme solution is stirred. Initial velocity is searched for from the absorbance change per for [of wavelength peculiar to each reduction type coenzyme] 1 minute.
[0044] (3) According to the relative activity measurement method of the substrate specificity above, it changed into the substrate in reaction mixture, and D-mannose of the same concentration, D-fructose, D-galactose, a mannitol, epi-inositol, and a silo inositol were measured. Reaction initial velocity over a myo inositol It is Table 2 about the enzyme activity in each substrate at the time of being referred to as 100. It is shown. K.p. The enzyme of that it is the dehydrogenase which the enzyme of 24 shares of TK (s) origin has singularity higher than the Cairo inositol to a myo inositol, and does not act on the Cairo inositol substantially, and the F.sp. 671-share origin is that it is an enzyme with high singularity at a myo inositol, and B.sp.No.3. It is clear that the enzyme's of the origin it is the dehydrogenase which acts on a myo inositol and the Cairo inositol.
[0045] As a substrate, D-mannose, D-fructose, D-galactose, a mannitol, D-Cairo inositol (above, the Wako Pure Chem make, Japan), a myo inositol, epi-inositol, and a silo inositol (above, sigma company make, the U.S.) were used.
[0046]
[Table 2]

基質特異性

菌株名	K. p. TK24	B. sp. No. 3	F. sp. 671
補酵素	NAD	チオNAD	NAD
ミオイノシトール	100 %	9 %	100 %
カイロイノシトール	1 %未満	100 %	18 %
サイロイノシトール	1 %未満	0 %	1 %未満
エピイノシトール	1 %未満	0 %	2 %
ガラクトース	1 %未満	0 %	1 %未満
フルクトース	0 %	0 %	1 %未満
マンノース	1 %未満	0 %	1 %未満
マンニトール	1 %未満	0 %	0 %

[0047] (4) Optimal pH Kp.TK24 origin; The pH10.0 neighborhood (substrate; myo inositol)
B. sp.No.3 Origin; the pH11.0 neighborhood (substrate; Cairo inositol)
F. The sp.671 origin; The pH11.0 neighborhood (substrate; myo inositol)
[0048] The aforementioned relative activity measurement method is used and they are 100mM(s) in reaction mixture. It changes to pH10.0 glycine buffer solution. 100mM tris buffers (pH 7.0-9.0) and 100mM(s) It measured using each buffer solution of the glycine buffer solution (pH 9.0-11.0).
[0049] (5) Molecular weight Kp.TK24 Origin; 75000**15000B.sp.No.3 Origin; 135000**10000F.sp.671 Origin; 40000**10000 [0050] TSK Gel G300SW (0.75phix600mm), 4+0.05% NaN3 of eluate;50mM phosphate buffer solution (pH7.5) +0.2MNa2SOs, and a molecular weight marker used oriental yeast company make (Japan). Chromatography equipment uses Shimazu equipment (Japan) and is UV280nm. And it detected in the activity measurement of a fraction. activity

measurement -- the Kp.TK24 origin -- and -- the enzyme of the F.sp.671 origin -- a myo inositol -- as a substrate -- using -- B.sp.No.3 The enzyme of the origin used the Cairo inositol as a substrate.
[0051] (6) thermal stability Kp.TK24 origin; -- the processing for 40 degrees C and 15 minutes -- almost -- It has 100% of residual activity.
B. sp.No.3 origin; -- the processing for 60 degrees C and 15 minutes -- almost -- It has 100% of residual activity.
F. the sp.671 origin ; the processing for 40 degrees C and 15 minutes -- almost -- It has 100% of residual activity.
[0052] Enzyme liquid and about 5U/ml Heat-treatment for 15 minutes was performed. Residual activity was measured by the aforementioned enzyme activity measuring method. Activity measurement uses a myo inositol as a substrate, and the enzyme of the Kp.TK24 and F.sp.671 origin is B.sp.No.3. The enzyme of the origin used the Cairo inositol as a substrate.
[0053] (7) Use the relative activity measurement method of Km value above, and it is the concentration and NAD of a myo inositol or the Cairo inositol. And thio NAD Concentration was changed and each Km value was measured. In addition, Kp.TK24 origin enzymes are 2mM(s). NAD It uses, Km value over a substrate is measured, Km value of a coenzyme is measured using 20mM myo inositol, and it is B.sp.No.3. The enzyme of the origin is 1mM. NAD It used, Km value over a substrate was measured, and Km value of a coenzyme was measured using 10mM myo inositol. Moreover, the F.sp. 671-share origin enzyme changed substrate concentration using said activity measurement method, and computed Km value.
[0054]
基質に対するKm値

ミオイノシトール	;	K.p.TK24 由来	; 49.6±5.0mM
		F.sp.671 由来	; 1.7±0.2mM
カイロイノシトール	;	B.sp.No.3由来	; 5.2±0.5mM

[0055]
補酵素に対するKm値

NAD	;	K.p.TK24由来	; 1.5±0.2mM
		B.sp.No.3 由来	; 0.5±0.1mM
		F.sp.671 由来	; 0.04±0.01mM
チオNAD	;	B.sp.No.3 由来	; 0.9±0.1mM
		F.sp.671由来	; 4.5±1mM

[0056] Although what kind of oxidase may be used as long as it is the oxidase which acts on a myo inositol as an oxidase which can be used for this invention, inositol oxygenase, a pyranose oxidase, etc. are raised, for example. Inositol oxygenase (510 Biochem.Biophys.Acta 167,501- 1968) And pyranose oxidase (500 Biochem.Biophys.Acta 167,493- 1968) It is well-known and well-known similarly about a purification method and a property.
[0057] Although what kind of kinase may be used as long as it is the kinase which acts on a myo inositol as a kinase which can be used for this invention, a myo inositol kinase etc. is raised, for example. The inositol kinase (562 Biochem.Biophys.Res.Commum 19,558- 1965) is well-known, and well-known similarly about a purification method and a property.
[0058] About the liquid presentation using the dehydrogenase which carries out the quantum of the myo inositol which can be used for this invention, the enzyme concentration which can be used chooses a coenzyme suitably one kind or more than it in consideration of Km value between 0.01-1000U/ml and the various coenzymes of the dehydrogenase which 0.05 - 500 U/ml is especially desirable, and is used etc., and it should just set up reaction mixture pH suitably so that a reaction may advance efficiently from the curve of optimal pH after that.
[0059] In measuring a myo inositol independently For example, that what is necessary is just to use the

dehydrogenase which acts on the inositol of 24 shares of above mentioned Kp.TK(s), or the F.sp. 371-share origin In using the dehydrogenase of the 24 shares of Kp.TK(s) origin, as a coenzyme although which coenzyme may be used as long as it is the coenzyme which can react -- suitable -- NAD it is -- for example, NAD receiving Km value -- 1.5mM(s) it is -- since -- Concentration of NAD 0.02M-500mM -- desirable -- 0.1-100mM it is . moreover -- since optimal pH is the ten neighborhoods -- pH of a reaction 8-12 -- especially -- 9-11 are desirable and the pH10 neighborhood where it becomes impossible for the operation especially over the Cairo inositol to check mostly in, and the enzyme activity over a myo inositol becomes max is the most desirable.

[0060] F. In using the dehydrogenase of sp. 371-share origin Although which coenzyme may be used as long as it is as a coenzyme with the coenzyme which can react suitable -- NAD Or thio NAD -- it is -- for example, -- Since Km values over NAD are 0.04mM(s) the concentration of NAD -- 0.005 -- M to 50 mM desirable -- 0.01-10mM -- it is -- thio Km value over NAD -- 4.5mM(s) it is -- since -- thio Concentration of NAD 0.02M-20mM -- desirable -- It is 0.1-10mM. moreover -- since optimal pH is the ten neighborhoods -- pH of a reaction 8-12 -- especially -- 9-11 are desirable.

[0061] About the enzyme reaction liquid presentation which carries out the quantum of a myo inositol and the Cairo inositol to coincidence as inositols For example, although which coenzyme may be used that what is necessary is just to use the enzyme of the B.sp.No3 origin as long as it is the coenzyme which can react as a coenzyme suitable -- NAD and thio NAD it is -- for example, NAD receiving Km value -- 0.5mM(s) it is -- since -- It mM(s). the concentration of NAD -- 0.01-100 -- It is 0.05-50mM preferably and is Thio NAD. Since it is 0.9mM(s), receiving Km value is Thio NAD. Concentration 0.02-100mM, desirable -- since it is 0.1-50mM and optimal pH is the 11 neighborhoods -- pH of a reaction 6-13 -- especially -- 8-12 are desirable.

[0062] Moreover, about the reaction mixture presentation in the case of measuring a myo inositol and the Cairo inositol to coincidence as inositols, when measuring said myo inositol and the Cairo inositol independently, a class, an amount, etc. of a sample which carry out the quantum of the inositol can determine suitably, and enzymes other than this and amounts other than this can also be used.

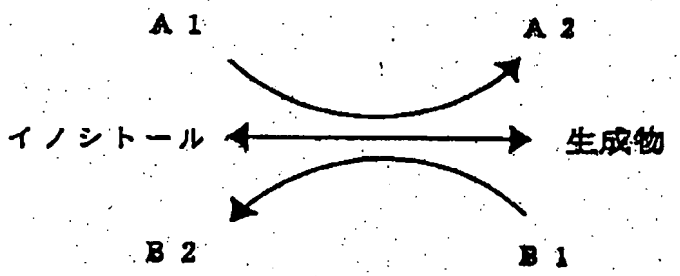
[0063] In measuring the Cairo inositol independently similarly For example, the above mentioned S.r.301 That what is necessary is just to use the inositol dehydrogenase of the stock origin Although which coenzyme may be used as long as it is with the coenzyme which can react as a coenzyme suitable -- NAD a kind (NAD, Acetyl NAD, and deamino NAD) -- it is -- NAD Concentration of a kind 0.02M-500mM -- desirable -- 0.1-100mM it is -- moreover -- since optimal pH is the nine neighborhoods -- pH of a reaction -- 7-11 -- especially 8-10 is desirable.

[0064] the enzyme concentration which can be used about the reaction mixture presentation in the case of using the oxidase which acts on a myo inositol -- 0.01-1000U/ml -- what is necessary is for 0.05 - 500 U/ml to be desirable, and just to set up reaction mixture pH suitably especially, so that a reaction may advance efficiently from the curve of optimal pH For example, that what is necessary is just to use the above mentioned inositol oxygenase or the above mentioned pyranose oxidase etc., in using a pyranose oxidase, optimal pH Since it is 6.0-8.0, it is pH of a reaction. 6.0-8.0 It is desirable.

[0065] the enzyme concentration which can be used about the reaction mixture presentation in the case of using the kinase which acts on a myo inositol -- 0.01-1000U/ml -- especially -- 0.05 - 500 U/ml -- desirable -- ATP required subsequently to a reaction etc. -- what is necessary is to set up the concentration of metal salts, such as a phosphate donor and magnesium, and just to set up reaction mixture pH suitably so that a reaction may advance efficiently from the curve of optimal pH for example, -- since the amount of the inositol in a living body is 10 or less mMs in general when using said inositol kinase carried out as the concentration of ATP -- 1-10mM -- desirable -- magnesium 1-30mM -- desirable -- optimal pH of an inositol kinase 6-9 it is -- since -- pH of a reaction 6-9 It is desirable. Moreover, in carrying out the quantum of the myo inositol, when high sensitivity is still more nearly required, the enzyme cycling method can be used. One of them is shown in a bottom type.

[0066]

[Formula 1]



[0067] the case where the inositol of the inside of a formula and a product is a myo inositol -- the MIOINO source 2 it is -- The compound with which the hydrogen atom of two atoms or four atoms was drawn out from the Cairo inositol when it was the Cairo inositol is shown. A1 shows NAD (P) or Thios NAD (P), A2 shows the reduction type of A1, B1 shows the reduction type thios NAD (P), when A1 is Thios NAD (P) and A1 is NAD (P) in reduction types NAD (P), and B-2 shows the oxidation type product of B1.

[0068] What is necessary is to choose a coenzyme suitably two kinds or more than it in consideration of Km value between the various coenzymes of the dehydrogenase which acts on the inositol to be used etc., and just to set up about the liquid presentation of the inositol quantum reaction using enzyme cycling, so that enzyme-cycling may advance pH conditions efficiently between the optimal pH of forward reaction/reverse reaction after that. The amount of A1 and B1 needs to be an excessive amount as compared with the amount of inositols in a sample, and to be an excessive amount even if it compares with Km value over A1 and B1 of the dehydrogenase which acts on an inositol.

[0069] B.sp.No.3 [for example,] if it sees about the inositol dehydrogenase of the origin -- Km value -- NAD and thio NAD ******** -- respectively -- 0.50 and 0.87mM -- small -- thios NAD and NAD It can consider as a coenzyme and can choose. Moreover, it is Thio NAD when performing a cycling reaction. Since it is the 10.5 neighborhoods and the optimal pH of reverse reaction using NADH is the ten neighborhoods, the optimal pH of the forward reaction at the time of using is good to choose Thios NAD and NADH as a coenzyme and to perform cycling in the pH10 neighborhood. the concentration of A1 and B1 -- 0.02mM-2M -- especially -- 0.05-100mM Amount of the enzyme which is desirable and acts on a myo inositol and the Cairo inositol 1-1000U/ml -- especially -- 5-500U/ml although it is desirable -- the amount -- a class, an amount, etc. of analyte -- suitably -- it can determine -- **** for amounts other than this -- things are also made.

[0070] Although it can perform the dehydrogenase independent which acts on an inositol as mentioned above, the enzyme cycling method is still more effective if it combines with the dehydrogenase which acts on other inositols from which the oxidase which acts on an inositol, and optimal pH differ.

[0071] moreover, in measuring the Cairo inositol and a myo inositol by high sensitivity alternatively Although what is necessary is to use an enzyme with high singularity for the Cairo inositol at the high enzyme and high myo inositol of singularity, and just to measure using the enzyme cycling method etc. It may measure using the enzyme with which singularity differs, and the amount of a myo inositol may be calculated using a formula from each measured signal. Moreover, what is necessary is to use an enzyme with high singularity for the Cairo inositol, to eliminate the Cairo inositol, and just to measure this reaction mixture by the high sensitivity measuring method using the enzyme which acts on a myo inositol.

[0072] The case where it measures by the enzyme cycling method, for example, using the enzyme of the B.sp.No3 origin and the enzyme of the F.sp.671 origin as an approach of measuring a myo inositol and the Cairo inositol alternatively using a formula is mentioned. It is thought that these enzymes have high singularity to an inositol in a biological material, and only the inositol is measured, and the signal of enzyme cycling is considered to be the sum of the signal obtained from a myo inositol, and the signal obtained from the Cairo inositol. For example, when a Cairo INOSHI roll and the standard solution of a

myo inositol are measured in the following reaction mixture using the enzyme of the B.sp.No3 origin, it is absorbance change mABS/min = 1.2x Cairo inositol concentration. (muM) Absorbance change mABS/min = 0.1x myo inositol concentration (muM) Since it becomes, when a biological material is measured in enzyme cycling using the enzyme of the B.sp.No3 origin, it is a bottom type (1). It is obtained.

[0073]

Absorbance change (B. sp.No3) mABS/min = 1.2x Cairo inositol concentration (muM) +0.1x myo inositol concentration (muM) (1) [0074] <Reaction mixture presentation> 100mM Glycine buffer solution pH9.82.0mM Thio NAD30microM NADH100U/ml Dehydrogenase which acts on an inositol (B. sp.No3 origin) [0075] When a Cairo INOSHI roll and the standard solution of a myo inositol are similarly measured in the following reaction mixture using the enzyme of the F.sp.671 origin, it is absorbance change mABS/min = 0.03 x Cairo inositol concentration. (muM) Absorbance change mABS/min = 0.2x myo inositol concentration (muM) Since it becomes, when a biological material is measured in enzyme cycling using the enzyme of the F.sp.671 origin, it is a bottom type (2). It is obtained.

Absorbance change (F. sp.671) mABS/min =0.03x Cairo inositol concentration (muM) +0.2 x myo inositol concentration (muM) (2) [0076] <Reaction mixture presentation> 100mM POPSO Buffer solution pH8.52.0mM Thio NAD30microM NADH4U/ml Dehydrogenase which acts on an inositol (F. sp.671 origin) [0077] Above (1) and (2) If the simultaneous equations of an equation are solved, the amount of a myo inositol is computable like a bottom equation.

Myo inositol concentration (muM) = 6.1x**mABS/min(F. sp.671)-0.1x**mABS/min (B. sp.No3)

[0078] Moreover, when the enzyme of the F.sp.671 origin is used, in the living body, it compares with the Cairo inositol and a myo inositol exists in large quantities. therefore, the Cairo inositol concentration (muM) << myo inositol concentration (muM) it is -- formula (2) Absorbance change (F. sp.671) mABS/min** 0.2x myo inositol concentration (muM) ** -- it can regard and myo inositol concentration can be measured to high sensitivity by this reagent independent.

[0079] Although the reaction which eliminates the Cairo inositol using an enzyme with high singularity can use any enzyme for the Cairo inositol if it is an enzyme with high singularity, the dehydrogenase, oxidase, and kinase which act on an inositol, for example are mentioned.

[0080] although which approach may be used as long as a deproteinization is the approach which can do a deproteinization as well as a well-known deproteinization method -- ethanol, an acetone, and TCA etc. -- the used approach and molecular weight 1 What is necessary is just to use the approach using about 10,000 fractionation film etc. What is necessary is to use an inositol dehydrogenase with high singularity for said Cairo inositol for a myo inositol, still more nearly alternative and in measuring to high sensitivity, to decompose, to carry out the deproteinization of nothing and the Cairo inositol for the Cairo inositol to Cairo INOSOSU 2, and just to measure a myo inositol using said enzyme cycling reagent. It is Cairo INOSOSU 2 about the Cairo inositol. The reaction to make The liquid presentation which measures said Cairo inositol independently can be used, and it is Cairo INOSOSU 2. The reaction to decompose Cairo INOSOSU 2 Although which approach may be used as long as it is the conditions which can be decomposed, it can decompose by oxidizer processing, heat treatment, alkali treatment, etc., and the simplest approach is heat treatment and should just perform 60 degrees C of processings for -15 minutes or more 50-degree C -15 minutes or more preferably. It is good to use an enzyme with high singularity for the Cairo inositol, and to perform the elimination reaction of the Cairo inositol.

[0081] the case where the oxidase and kinase which act on a myo inositol are used -- for example, the Cairo inositol oxygenase -- the Cairo inositol -- glucuronic acid -- change **** -- if the Cairo inositol oxygenase is made to react with a sample and the inositol in this reaction mixture is measured from things to high sensitivity using the enzyme cycling method etc., a myo inositol can be measured specifically. Similarly, from changing the Cairo myo inositol to Cairo inositol 1 phosphoric acid, the Cairo inositol kinase can measure a myo inositol specifically, if the inositol in this reaction mixture is similarly measured to high sensitivity using the enzyme cycling method etc.

[0082] Furthermore, the quantum of the inositols in a sample can be carried out to coincidence using

enzyme cycling, on the other hand, the quantum of the Cairo inositol in a sample can be independently carried out to high sensitivity using the aforementioned approach, and the amount of myo inositols can also be calculated from the difference.

[0083] When using a dehydrogenase, detection of a myo inositol moreover, the variation of a coenzyme For example, it is NAD as a coenzyme. Reduction type NAD which is a reduction type coenzyme as an amount of change used and generated 340nm which is the absorption maximum wavelength region [whether a direct quantum is carried out using well-known techniques, such as measuring with a colorimeter, on neighboring wavelength, and] The reduction type coenzyme produced, for example Or various diaphorases or a phenazine methosulfate (it omits Following PMS), Methoxy PMS the various tetrazolium salt represented by an electronic carrier and nitro tetrazolium, such as dimethylaminobenzophenoxadinium chloride (Mel Drabble), -- water solubility is preferably high Whether a quantum is indirectly carried out using reduction system color reagents, such as WST-1-8 (member chemistry company make) Using the combination of a dehydrogenase and an oxidase suitably, a hydrogen peroxide is generated from a reduction type coenzyme, and the hydrogen peroxide to produce may be measured directly and indirectly.

[0084] The amount of the above-mentioned hydrogen peroxide may generate coloring matter etc. for example, using a par oxidase etc., it may carry out a quantum according to luminescence fluorescence etc., and it may carry out the quantum of the amount of the aldehyde which could carry out the quantum, was made to generate an aldehyde and was produced from alcohol again using the catalase etc. by the electrochemical technique. The direct oxidation and leuco mold reagent which carry out coloration can be used for the coloring system of a hydrogen peroxide under existence of the TORINDA reagent which generates coloring matter under existence of a par oxidase by the oxidation condensation of couplers, such as 4-AA or a 3-methyl-2-benzothiazolinone hydrazone (MBTH), and chromogens, such as a phenol, and a par oxidase.

[0085] As a chromogen of a TORINDA mold reagent, a phenol derivative, an aniline derivative, A toluidine derivative etc. is usable. As an example N, N dimethylaniline, N and N-diethylaniline, 2, 4-dichlorophenol, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxy aniline (DAOS), N-ethyl-N - Sulfopropyl -3, 5 dimethylaniline (MAPS), N-ethyl-N-(2-hydroxy-3- sulfopropyl)-3, 5-dimethylaniline (MAOS), N-ethyl-N -(2-hydroxy-3- sulfopropyl)- Meta toluidine (TOOS), N-ethyl-N-sulfopropyl - m - Anisidine (ADPS), N-ethyl-N - A sulfopropyl aniline (ALPS), N-ethyl-N - Sulfopropyl -3, 5-dimethoxy aniline (DAPS), N-sulfopropyl -3 and 5-dimethoxy aniline (HDAPS), N-ethyl-N - Sulfopropyl - m - Toluidine (TOPS), An N-ethyl-N-(2-hydroxy-3- sulfopropyl)-m-anisidine (ADOS), An N-ethyl-N-(2-hydroxy-3-sulfopropyl) aniline (ALOS), N-(2-hydroxy-3- sulfopropyl)-3 and 5-dimethoxy aniline (HDAOS), an N-sulfopropyl-aniline (HALPS) (above, member national-chemical-laborator company make), etc. are mentioned.

[0086] Moreover, as an example of a leuco mold reagent, it is o-dianisidine, ortho tolidine, and 3 and 3. It is member national-chemical-laborator company make, N--(carboxymethyl aminocarbonyl) 4, and 4-bis(dimethylamino) biphenyl amine (DA64), 10-(carboxymethyl aminocarbonyl)-3, and 7-bis (dimethylamino) phenothiazin (DA67) diaminobenzidine, 3, 3 and 5, and more than 5-tetramethyl benzidine;.; The Wako Pure Chem make etc. is mentioned above.

[0087] Furthermore, the compound which emits fluorescence by oxidization to a fluorescence method, for example, homovanillic acid, 4-hydroxyphenyl acetic acid, tyramine, the Parakou resol, a diacetyl full ORESUSHIN derivative, etc. can be used for a chemiluminescence method for luminol, lucigenin, iso luminol, pyrogallol, etc. as a catalyst. Using a catalase etc., an aldehyde is made to generate from alcohol and the approach using a Hantzsch reaction as an approach of carrying out the quantum of the produced aldehyde, the approach of making it color by the condensation reaction with MBTH or the approach using aldehyde dehydrogenase, etc. is mentioned.

[0088] What is necessary is just to carry out the quantum of the quantum of the glucosone using well-known aldose reagents, such as a diphenylamine. What is necessary is just to measure glucuronic acid using well-known approaches, such as an orcinol reaction, since glucuronic acid is produced that what is necessary is it to be desirable to measure the consumption of oxygen or the amount of a resultant when

using an oxidase, and for a hydrogen peroxide and the glucosone to generate and just to measure a hydrogen peroxide and the glucosone using the aforementioned approach when a pyranose oxidase is used for the amount of a resultant as measurement, for example when inositol oxygenase is used.
[0089] the phosphoric acid object of the myo inositol which it produces from a reaction in using a kinase -- or -- It is desirable to measure the amount of ADP. ADP It is the approach and ADP using the approach, pyruvate kinase, and pyruvate oxidase using a pyruvate kinase well-known as a measuring method, and lactate DENIDOROGENAZE. It can measure using the approach (Japanese Patent Application No. No. 340482 [07 to]) using the hexokinase to be used etc.
[0090] Moreover, detection of a myo inositol may fix in an electrode the enzyme which acts on a myo inositol, and may detect it electrochemically. For example, what is necessary is to carry out direct electrode measurement of the reduction type coenzyme similarly, or to make an electron carrier intervene, and just to measure the oxidation reduction current acquired or its quantity of electricity that what is necessary is to measure the produced hydrogen peroxide with a direct electrode, or to make electron carriers, such as a ferrocene derivative or a quinone derivative, intervene when using an oxidase, and just to measure the oxidation reduction current acquired or its quantity of electricity, also when using a dehydrogenase.
[0091] In this way, with the prepared constituent for inositol quanta, in order to carry out the quantum of the inositol in a sample It is a sample to the constituent for inositol quanta. 0.001-0.5ml In making it react at the temperature of 37 degrees C and performing rate assay in addition For several minutes between two after fixed time amount after reaction initiation thru/or dozens of minutes (for example, 3) For [of a part and 4 minutes after] 1 minute, Or what is necessary is just to measure directly or indirectly the amount of the coenzyme from which it changed after after [reaction initiation] fixed time amount that what is necessary is just to measure directly or indirectly the amount of the coenzyme for 5 minutes of 8 minutes after which changed the 3 whole minutes in the case of end point assay. In this case, if it compares with change of the absorbance at the time of measuring using the myo inositol or the Cairo inositol of known concentration etc., the amount of the myo inositol in a sample or the Cairo inositol can be calculated. In addition, a quantum can also be performed instead of spectrometry using other well-known measuring methods in measurement of the amount of oxygen which changed in this invention, the amount of hydrogen peroxides, the amount of coenzymes, and the amount of a product.
[0092] What is necessary is finally, to carry out the quantum of the myo inositol in a sample by the aforementioned approach, and just to judge with their being a diabetes-mellitus reserve group or diabetes mellitus in beyond the characteristic value of a normal type and a normal type, when the acquired quantum value is under a characteristic value of a normal type in order to judge a normal type and a diabetes-mellitus reserve group. What is necessary is just to remove diabetes mellitus from a diabetes-mellitus reserve group or diabetes mellitus, in order to distinguish only a diabetes-mellitus reserve group. A diabetic judgment is WHO in a well-known approach, for example, a glucose tolerance test. Or it sorts out by the decision criterion shown in 1998, it is ******HbA1c to the Japanese old ** method. Distinguish with a value. In addition, judge with blood sugar markers, such as fructosamine, the Glico albumin, and a 1.5 anhydro KISHIGURUSHI toll. He is WHO by the fasting blood sugar level. Which approach may be used, as long as the approach of sorting out by the decision criterion shown in 1998 etc. is mentioned and it can perform a diabetic judgment also except this.
[0093] Although you may be which group as long as the diabetes-mellitus reserve group in this invention is a high group of the establishment which will shift to diabetes mellitus among non-diabetes-mellitus persons in the future, a boundary mold, IFG, IGT, or insulin resistance is mentioned, for example. A boundary mold points out the boundary mold defined in Japan Diabetes Society in 1999, and is 75gOGTT here. When it carries out, what does not belong to a diabetes-mellitus mold (126 or more mg/dl of fasting blood sugar levels, 200 or more mg/dl of after [a load] 2-hour blood sugar) and a normal type (less than 110 mg/dl of fasting blood sugar levels, less than 200 mg/dl of after [a load] 2-hour blood sugar) is shown. Again IFG and IGT WHO IFG of the decision criterion shown in 1998 And IGT It is shown and is 75gOGTT. It is IFG when it carries out. Fasting blood sugar level It is 110 - 125 mg/dl and less than 140 mg/dl of after [a load] 2-hour blood sugar, and is IGT. Less than 126 mg/dl of

fasting blood sugar levels, after [a load] 2-hour blood sugar It is 140 - 200 mg/dl. incidentally -- boundary mold =IFG+IGT it is .
[0094] Although urine and urine collection can be used at any time when using urine for a sample, it is desirable to ** by creatinine concentration and to convert into the inositol concentration in a fixed concentration creatinine. However, since the case where a creatinine value becomes unusual in the patient who has kidney disease is also considered, as for the sample a creatinine indicates outlying observation to be, excepting from inspection is desirable. Moreover, since the inositol in urine is strongly influenced of the resorption in the kidney etc., it can inspect a diabetes-mellitus reserve group to high rate more by excepting kidney disease (the patient who presents the micro albuminuria etc., or patient of nephrogenic urine sugar).
[0095] moreover, the group whose above-mentioned myo inositol is beyond the characteristic value of a normal type when judging a diabetes-mellitus reserve group by the high probability -- in addition, a fasting blood sugar level, glycosylated hemoglobin, and saccharification -- a diabetes-mellitus reserve group or diabetes mellitus can be judged by the higher probability by adding the group beyond the characteristic value of the normal type of albumin.
[0096]
[Embodiment of the Invention] Although the example and the example of reference of this invention are described in detail, thereby, this invention is not limited at all.
[The example 1 of reference] (GC/MASS Recovery test of law and enzymatic process) Myo inositol (sigma company make) concentration is 0, 10, 20, 30 and 40, and 50microM to a blood serum in a blood serum. What was prepared so that it might become was used as a sample.
[0097] The <GC/MS method> 1 Each blood serum of the pretreatment above of a sample 100microL Except for [a 1mg urease is added, and it incubates for 30 minutes at 37 degrees C, and] a urea. Subsequently, it is n-heptadecanoic acid 20microg as internal standard matter. A D7-glucose is added and it is 1mL. Centrifugal separation of the dehydrated ethanol (the Wako Pure Chem make, best) was added and carried out, it carried out the deproteinization, and concentration hardening by drying was carried out by the evaporator under reduced pressure of the upper layer. Further 100microL BSTFA (N, O - screw trimethylsilyl TORIFURORO acetamido, Wako Pure Chem make) and 10microL TMCS (trimethylchlorosilane, Funakoshi) is added, and it heats for 30 minutes at 80 degrees C, and is TMS. It considered as the derivative.
[0098] 2) GC/MS The analysis quadrupole mold Automass system (JEOL) is used, and it is GC/MS. It analyzed. GC is Ultra Alloy plus-5+. The gaseous helium of rate-of-flow 1.47 mL/min was passed as carrier gas to the metal capillary column (30mx0.25mm i.d., 0.25micrometer filmthickness, Frontier Lab.KK), and was analyzed to it. Sample 2microL Automatic impregnation is carried out in 38:1 split mode, and it is from 60 degrees C. Temperature up analysis was carried out by 17 degrees C / min to 350 degrees C. a mass spectrum -- electron impact (EI) -- law From m/z50 up to m/z650 a 0.4-second scan -- low -- resolution -- it measured in the mode.
[0099] <Enzymatic process> 1 Reagent <R-1 ; Glucose elimination reagent >10mM Tris-buffers 60mM Magnesium chloride (Wako Pure Chem make)
88mM(s) ATP (oriental yeast company make)
43U/ml Hexokinase II (Asahi Chemical Industry Co., Ltd. make)
40mM(s) Oxalic acid (Wako Pure Chem make)
6mM(s) Thio NAD [0100] <R-2 ; Myo inositol quantum reagent >200mM Glycine buffer solution (pH 9.8)
2mM(s) Thio NAD (oriental yeast company make, Japan)
0.03mM(s) NADH (oriental yeast company make, Japan)
100U/ml B.sp.No.3 Myo-inositol dehydrogenase of the stock origin [0101] 2) each blood serum of the actuation above 100microL Glucose elimination reagent 50microl adding -- 37 degree-C-5 a part -- between -- a glucose elimination reaction -- carrying out -- subsequently -- myo inositol quantum reagent 100microL In addition, the reaction was started. 1 minute after reaction initiation, and 405/3nm The absorbance which can be set was read, the difference was taken and it asked for the absorbance

change around for 1 minute.
[0102] 3) Result GC/MS The recovery test result of the myo inositol by law and enzymatic process is shown in Table 3. GC/MS Although addition recovery of the myo inositol by law was poor, the addition recovery by enzymatic process was very good.
[0103]
[Table 3]

添加回収試験結果

ミオイノシトール(μM)	0	10	20	30	40	50
GC／MS法		60%	40%	40%	103%	94%
酵素サイクリング法	—	104%	105%	98%	101%	100%

[0104]
[The example 2 of reference] (Culture of a fungus body, and purification of an enzyme)
1) Culture and the purification yeast extract of Klebsiella pneumoniae (Klebsiella pneumoniae) TK24 (FERM BP-6506) 2%, Peptone 2% (above the Far East Pharmaceuticals company make, Japan), glycerol 2%, phosphoric acid [ ] -- 0.1% (domestic chemistry company make --) Two potassiums 0.1% (above the Wako Pure Chem make, Japan) and glucose Japan and pH7.0 100ml of included liquid media 500ml It pours distributively to an Erlenmeyer flask. After heat-sterilizing by 121 ** for 20 minutes, it is 1 of 24 shares (FERM BP-6506) of Klebsiella pneumoniae (Klebsiella pneumoniae) TK to this. A platinum loop is inoculated. It cultivated with the shaking-culture vessel of 120rpm at 28 degrees C for 20 hours, and 85ml (activity 0.04U/ml) of **** was obtained.
[0105] the medium composition same on the other hand as the above -- as a defoaming agent -- De Dis form BC51Y (the Nippon Oil & Fats Co., Ltd. make --) Liquid-medium 20L which did 0.1 % addition of Japan 30L After teaching the ** jar fermenter and sterilizing after heating, 85ml of above **** is transplanted. Aeration culture is carried out for 16 hours by part for the culture temperature of 30 degrees C, and quantity-of-airflow 20L/, the internal pressure of 0.4kg/cm2, and agitating speed 200rpm, and it is culture 20L (enzyme activity 0.12U/ml). It obtained.
[0106] The harvest of the obtained culture is carried out by centrifugal separation, and they are 10mM tris buffers about this. pH8.0 was distributed, and it ultrasonicated, cooling by the ice bath, and the fungus body was solubilized. Solubilization liquid is 3000rpm15. Centrifugal separation of a part was performed and solubilization supernatant liquid 2.5L (2U/ml) was obtained.
[0107] It is the 10mM phosphate buffer pH 7.5 about this enzyme liquid. Equilibrated Q-sepharose (Sepharose) B.B.(Pharmacia biotechnology tech-harmacia Biotech shrine make, Sweden country) 1.25L It applied and fraction 2.2L (1.5U/ml) which has elution and activity in step WAIZU with 0, 0.1, 0.2, and the same buffer solution containing KCl (Nakarai Tesuku; the product made from nacalai tesque, Japan) of 0.3M was obtained. The obtained enzyme liquid adds NaCl (the Nakarai Tesuku make, Japan) so that it may be set to 4M, and it is 4M NaCl. Phenyl sepharose FF200(Pharmacia manufacture, Sweden country) ml equilibrated with included 10mM phosphate buffer solution (pH7.5) It applies and is 4 ->0 M. Expansion and an activity peak are pooled in a NaCl gradient, and it is 600ml of enzyme solutions. It obtained. The obtained enzyme solution is 10mM phosphate buffer solution pH7.5. It receives and dialyzes and is molecular weight 5. It condenses by the film of 10,000 cuts. The 100ml (27U/ml) enzyme solution was obtained. The obtained enzyme liquid was frozen and was saved at -20 degrees C.
2) Culture and purification were performed by the same approach as the examples 1 and 2 of reference opened to culture and purification WO No. 42863 [98 to] of Bacillus sp. (Bacillus sp.) No.3 (FERM BP-5881).
3) Culture and purification 1 of Flavobacterium ESUPI (flavobacterium sp.) 371 (FERM BP-7323) Culture and purification were performed by the same approach as culture of Klebsiella pneumoniae and

purification.
[0108]
[The example 3 of reference] (Quantum of the specific inositol using an enzyme)
1) Myo inositol quantum reagent 100mM Glycine buffer-solution (pH 10.0) 2mM Oxidation type NAD (oriental yeast company make, Japan)
5U/ml Kp.TK24 Myo-inositol dehydrogenase 2 of the stock origin The 1ml of the actuation aforementioned quantum reagents 0.02ml of water solutions which contain a myo inositol (sigma company make) 0, 50,100, 150, and 200mM at 37 degrees C for a cuvette -- in addition, 37 degrees C and the reaction for 10 minutes were performed. It lets a reaction pass and is 340nm. It measured, and from the absorbance for 10 minutes after substrate water-solution addition, the absorbance before substrate water-solution addition was deducted, and it asked for absorbance change. The result is shown in drawing 1 . the quantum only of the myo inositol is carried out simple by using an enzyme with high singularity for a myo inositol -- clear -- moreover, this substrate concentration field -- setting -- the conversion rate of a substrate -- almost -- It was 100% and was possible at ideal end point measurement.
[0109]
[The example 4 of reference] (Quantum of the myo inositol using the oxidase which acts on a myo inositol)
1) Myo inositol quantum reagent 100mM Tris-buffers (pH7.0) 100 U/mL Pyranose oxidase 5 U/mL POD (par oxidase sigma company make)
1.5 MuMol/mL 4-AA (Wako Pure Chem Make)
4.5 MuMol/mL TOOS (Wako Pure Chem Make)
[0110] 2) Actuation above-mentioned reagent 3mL Water-solution 80microL which takes to a cuvette and contains a myo inositol (sigma company make) 0.2, 2, 10 and 15, and 20mM It added and the reaction was started at 37 degrees C. It lets a reaction pass and is 515nm. It measured, the substrate water-solution additive-free absorbance was deducted from the absorbance for 5 minutes after substrate water-solution addition, and it asked for absorbance change. The result is shown in drawing 2 . By using the oxidase which acts on a myo inositol, the quantum of the myo inositol was able to be carried out simple.
[0111]
[The example 5 of reference] (Quantum of the myo inositol using the kinase which acts on a myo inositol)
1) Myo inositol quantum reagent 50mM Tris buffers (pH7.5) 2mM ATP (oriental yeast company make)
10mM(s) Magnesium chloride (Wako Pure Chem make)
10 U/mL Myo inositol kinase (Science 151,198-199 (1966))
10mM(s) Glucose (Wako Pure Chem make)
10 U/mL ADP-HK (Asahi Chemical Industry Co., Ltd. make)
1mM NADP (oriental yeast company make)
5 U/mL Glucose-6-phosphoric-acid dehydrogenase (Toyobo Co., Ltd. make) [0112] 2) Actuation above-mentioned reagent 1.0mL It took to the cuvette, a myo inositol (sigma company make) 0 and 100micro of water solutions L containing 50,100,150,200microM were added, and the reaction was started at 37 degrees C. After reaction initiation, 0 minute, and 340/5nm Absorbance change which can be set was read and the difference was searched for. A result is shown in drawing 3 . As shown in drawing 3 , the quantum of the myo inositol was able to be carried out simple by using the enzyme which acts on a myo inositol.
[0113]
[The example 6 of reference] (Quantum of the high sensitivity myo inositol using an enzyme)
1) Myo inositol quantum high sensitivity reagent 100mM POPSO buffer-solution (pH 8.5) 2mM Thio NAD (oriental yeast company make, Japan)
0.03mM(s) NADH (oriental yeast company make, Japan)
3.5U/ml Myo-inositol dehydrogenase of the F.sp. 671-share origin [0114] 2) The 1ml of the actuation above-mentioned reagents For a cuvette, they are 0, 5, 10, 20, 30 and 40, and 50microM. Myo inositol

(sigma company make) Solution 20microl It added and the reaction was made to start at 37 degrees C. 1 of reaction time A part and 3 It is 405nm to eye a part. The absorbance which can be set is read, the difference is taken and it is 1. It asked for the absorbance change around between parts. The result is shown in drawing 4 . The absorbance change to the amount of myo inositols showed the good straight line, and high sensitivity measurement of the myo inositol using enzyme cycling was possible for it as shown in drawing 4 . Moreover, this enzyme had singularity higher than the Cairo inositol to the myo inositol, and it became clear that a myo inositol can be measured in the living body, without pretreating a sample alternatively, when it measured biogenic substances (a blood serum, plasma, urine, etc.), since the myo inositol existed in large quantities compared with the Cairo inositol.

[0115]

[Example 1] (Judgment of the diabetes-mellitus reserve group by the myo inositol measurement in a sample)

Specimen; blood serum (sample of the candidate who is not diabetes mellitus)

The classification of a diabetes-mellitus condition; the judgment of a normal type and a boundary mold will be based on the criteria of revised Japan Diabetes Society in said 1999, and is normal, IGT, and IFG. A judgment is WHO in 1998. It carried out based on criteria.

[0116] Myo inositol measurement reagent; it is the same as the example 1 of reference.

Fasting blood sugar level; it measured using glucose II-HA Test Wako (Wako Pure Chem make).

the time of hungry -- insulin value; -- it measured using Insulin (****) (Denka Seiken Co., Ltd. make) EIA.

A HOMA; fasting blood sugar level (mg/dl) x insulin value (muU/ml) / 400 glycosylated hemoglobin; it measured using the first science company make of glycosylated hemoglobin meter (high OTOE one C HA-8150) Kyoto.

saccharification -- albumin; -- it measured using the first science company make of Glico albumoscope (GAA-2000) Kyoto.

Fructosamine; it measured using auto WAKO fructosamine (Wako Pure Chem make).

[0117] The inositol in a blood serum was mainly a myo inositol, and since the Cairo inositol was considered to be below limit of detection, it performed the quantum using the calibration curve of [Richard E Ostland, Jr, Proc.Natl.Acad.Sci.USA, Vol.90 (1993) 9988-9992], and a myo inositol. Although a result is shown in drawing 5 , they are a boundary mold and IFG. And IGT A myo inositol quantum value is 0.0001% of level of significance. 27micromol/L which shows a high price intentionally below and is equivalent to average + standard deviation (average = 20.1micromol/L, standard deviation =6.8 mumol/L) as a characteristic value of a normal type They are a boundary mold and IFG above effectively at 80% of sensibility. And IGT It was able to judge. Moreover, you may transpose to average +2 x standard deviation and average +3 x standard deviation as a characteristic value of a normal type. Moreover, a boundary mold and IFG And IGT HOMA which insulin resistance is the cause in many cases, and is the index of insulin resistance above myo inositol quantum value mol/L of 27micro of a sample is also high (average =2.23, standard deviation = 0.32), and it is myo inositol quantum value mol/L of 27micro. At the following, HOMA can also judge insulin resistance by the quantum of a myo inositol low (average =1.77, standard deviation = 0.29).

[0118] The detection sensitivity of the boundary mold at the time of adding the judgment by the diabetes-mellitus marker of further others to the judgment by the myo inositol and singularity are shown in Table 4. When the judgment by the other diabetes-mellitus marker was added, diabetic detection sensitivity went up, as shown in Table 4. Setting up the cut-off value at this time with the average + standard deviation of a normal type, the set point is fasting blood sugar level 110 mg/dl (average 99.5 mg/dl, standard deviation =9.0 mg/dl) and HbA1c. Value 5.3 % (average 5.0 %, standard deviation =0.3 %), fructosamine value They were 220micromol/L (average 206micromol/L, standard deviation =14micromol/L) and 15.0% of the Glico albumin values (14.2% of averages, standard deviation =0.8 %).

[0119]

[Table 4]

ミオイノシトール単独及び他糖尿病マーカーによる判定を加えた場合の境界型検出の特異性及び感度

	境界型検出における	
	特異性	感度
ミオイノシトールのみ	95%	73%
ミオイノシトール＋空腹時血糖	90%	82%
ミオイノシトール＋ヘモグロビンA1c	81%	82%
ミオイノシトール＋フルクトサミン	86%	82%
ミオイノシトール＋グリコアルブミン	76%	82%

判定に用いたカットオフ値は下記の値を用いた。

ミオイノシトール ;正常型の平均値＋標準偏差＝27μM
空腹時血糖値 ;健常者の平均値＋標準偏差＝110mg/dL
糖化ヘモグロビンA1c ;健常者の平均値＋標準偏差＝5.3%
フルクトサミン値 ;健常者の平均値＋標準偏差＝220μM
グリコアルブミン値 ;健常者の平均値＋標準偏差＝15.0%

[0120]
[Example 2] Specimen; (Judgment of the diabetes-mellitus reserve group by the myo inositol measurement in a sample) Blood serum (sample extracted 2 hours 1 hour after enforcement and after enforcement from the candidate who enforced 75g glucose tolerance test at the time (before enforcement) of hungry)
Urine (sample extracted 2 hours 1 hour after enforcement and after enforcement from the candidate who enforced 75g glucose tolerance test at the time (before enforcement) of hungry)
The classification of a diabetes-mellitus condition; the judgment of a normal type, a boundary mold, and a diabetes-mellitus mold will be based on the criteria of amendment Japan Diabetes Society in said 1999, and the judgment of a normal type, IFG, IGT, and a diabetes-mellitus mold is WHO in 1998. It carried out based on criteria.
Myo inositol measurement reagent; it is the same as the example 6 of reference.
Blood sugar; it measured using glucose II-HA Test Wako (Wako Pure Chem make). Creatinine; it measured using creatinine-HA Test Wako (Wako Pure Chem make).
[0121] Since the inositol in urine was mainly a myo inositol, it performed the quantum using the calibration curve of a myo inositol. It **(ed) by the creatinine which carried out the quantum of the myo inositol in the urine 2 hours 1 hour after [before enforcing 75g glucose tolerance test (at the time of hungry)] enforcement, and after enforcement, and carried out the quantum to coincidence, and converted into the myo inositol concentration in a fixed concentration creatinine. Although a result is shown in drawing 6 and Table 5, a myo inositol quantum value is compared [in / on before OGTT enforcement, and / IFG, IGT, and a diabetes-mellitus mold] with a normal type, and it is level of significance, respectively. It reaches 0.02% 0.001%. 0.001% or less showed the high price intentionally. 10.9micro more thang/mg which is equivalent to average + standard deviation (average = 9.1 mug/mg, standard deviation =1.8 mug/mg) as a characteristic value of a normal type -- effective -- IFG, IGT, and a diabetes-mellitus mold -- respectively -- 100% and 80% -- and -- It was able to judge by 100% of sensibility. Moreover, as a characteristic value of a normal type, you may transpose to average +2x standard deviation and average +3 x standard deviation.
[0122]
[Table 5]

75g OGTT施行前後の尿中ミオイノシトール／クレアチニンの平均値、及び標準偏差

	75g OGTT施行前				75g OGTT施行1時間後				75g OGTT施行2時間後			
	正常型	IFG	IGT	糖尿病型	正常型	IFG	IGT	糖尿病型	正常型	IFG	IGT	糖尿病型
平均値	9.1	23.9	25.6	28.4	10.5	65.5	54.4	97.5	10.1	66.9	84.5	169.6
標準偏差	1.8	9.3	18.9	12.4	3.1	44.3	43.9	65.1	3.1	47.9	73.0	73.1
平均値＋標準偏差	10.9	33.3	44.6	40.8	13.6	109.8	98.3	162.6	13.1	114.8	157.6	242.6
平均値－標準偏差	－	14.6	6.7	16.0	－	21.2	10.6	32.4	－	19.0	11.5	96.5
危険率 ： p	－	0.0003	0.0169	0.0002	－	0.0019	0.007	0.0007	－	0.0027	0.0061	0

p ： 正常型に対する独立2群間のt検定での危険率

[0123] Although the amount of myo inositols excreted in the urine 1 hour after OGTT enforcement and of 2 hours after furthermore hardly changed compared with enforcement before with a normal type, compared with enforcement before, it went up greatly in IFG, IGT, and a diabetes-mellitus mold. Therefore, it is after [of OGTT enforcement] 2 hours. In IFG, IGT, and a diabetes-mellitus mold, the myo inositol quantum value was compared with the normal type, and showed the high price intentionally at level-of-significance 0.003 %, 0.01%, and 0% or less, respectively. 13.2micro more thang/mg which is equivalent to average + standard deviation (average = 10.1microg [// mg] and standard deviation =3.1 mug/mg) as a characteristic value of a normal type -- effective -- IFG, IGT, and a diabetes-mellitus mold -- respectively -- 100% and 93% -- and -- It was able to judge by 100% of sensibility. Moreover, you may transpose to average +2x standard deviation and average +3x standard deviation as a characteristic value of a normal type. Before and after OGTT enforcement, the sensibility the direction after enforcement judges a diabetes-mellitus reserve group to be was rising. It cannot be overemphasized by using this inspection approach from these results that a diabetes-mellitus reserve group, for example, IFG, IGT, a diabetes-mellitus mold, or insulin resistance can be detected to high sensitivity, without performing glucose tolerance tests, such as OGTT.

[0124]

[Example 3] (Correlation of 75g OGTT 2-hour blood sugar ground value and the myo inositol measured value in urine) Specimen; When fasting blood sugar was measured, OGTT was enforced in the group which was 110 or more mg/dl.

; Blood serum (sample extracted from the candidate who enforced 75g of glucose tolerance tests 2 hours 1 hour after time [of hungry] (before enforcement), and enforcement , and after enforcement); Urine (sample extracted from the candidate who enforced 75g of glucose tolerance tests 2 hours time [of hungry] (before enforcement), and 1 hour after enforcement and after enforcement) Measurement reagent of a myo inositol; It is the same as the example 6 of reference.

GC/MS of a myo inositol Analysis; it is the same as the example 1 of reference.

Blood sugar, a creatinine; it is the same as an example 2.

Count; the myo inositol in urine was measured with the reagent of the example 6 of reference, and computed myo inositol concentration from the value of a reference standard.

Result; correlation of the blood sugar level in 2 hours at the time of 75g OGTT enforcement and the myo inositol value in urine is shown in drawing 7 and Table 6. It is a correlation coefficient between the blood sugar level [in / when the enzyme cycling method is used / 2 hours at the time of 75g OGTT enforcement], and the myo inositol value in urine. High correlation of r= 0.70 was accepted. However, on the other hand, when the GC/MS method was used, correlation was not accepted between the blood sugar level in 2 hours at the time of 75g OGTT enforcement, and the myo inositol value in urine. Moreover, it is IGT so that drawing 7 may show. Criteria, the 2-hour blood sugar level at the time of 75g OGTT enforcement especially considered to be related to abnormal glucose tolerance 140 mg/dl The corresponding myo inositol value in urine is 80microg [/mg] creatinine, and it became clear from the confidence limit that it was a value with desirable 72-90microg [/mg] creatinine 68-94microg [/mg] creatinine from 95% confidence limit of the straight line obtained from the straight-line approximate expression, and 80 more%. When 80microg [/mg] creatinine which is the median is made into a cut-off value, it is the 2-hour blood sugar level at the time of 75g OGTT enforcement. 140 mg/dl 96% is contained under in a cut-off value, and the following is the 2-hour blood sugar level at the time of 75g OGTT enforcement. 140 mg/dl As for the above, 74% or more was contained beyond the cut-off value. It considers as the aforementioned characteristic value from this result, and also is the 2-hour blood sugar level at the time of 75g OGTT enforcement. 140 mg/dl It became clear that a considerable **** myo inositol quantum value could be made into a characteristic value. Moreover, since the 2-hour blood sugar level of 75g OGTT which shows extent of abnormal glucose tolerance, and the amount of myo inositols in urine had correlation, extent of abnormal glucose tolerance has been judged by measuring the myo inositol in urine using enzyme cycling.

[0125]

[Table 6]

実施例3に基づくOGTT施行前後の尿中ミオイノシトール

	血糖(mg/dL)		酵素サイクリング法 ミオイノシトール/クレアチニン(μg/mg)			GC/MS法 ミオイノシトール/クレアチニン(μg/mg)		尿糖/クレアチニン(mg/mg)		
	0時間	2時間	0時間	1時間	2時間	0時間	2時間	0時間	1時間	2時間
正常型	108.0	108.3	15.2	18.3	21.8	5.5	16.4	0.0	0.1	0.1
	107.2	116.5	34.6	35.9	49.7	21.9	65.1	0.1	0.1	0.1
	104.5	112.7	16.7	17.4	16.4	16.7	47.3	0.1	0.1	0.1
	88.1	70.3	9.7	8.5	12.3	4.2	33.0	0.0	0.1	0.1
	87.2	89.8	11.1	11.2	9.3	5.6	23.7	0.0	0.1	0.1
	96.8	104.9	19.3	28.9	24.0	15.1	36.7	0.0	0.1	0.0
	97.3	119.3	14.2	17.1	16.8	12.3	54.8	0.0	0.1	0.1
	90.0	81.4	9.4	9.2	11.7	3.8	48.4	0.0	0.1	0.1
	100.6	79.1	7.7	8.3	7.6	7.6	28.0	0.0	0.1	0.1
	95.2	78.3	12.7	41.8	28.0	3.4	26.1	0.1	3.8	1.1
	87.9	96.6	14.8	11.7	9.9	4.8	14.0	0.0	0.1	0.1
	90.8	83.6	18.6	21.2	26.8	9.3	11.0	0.1	0.1	0.2
	93.8	78.7	11.0	10.7	10.2	16.6	15.8	0.1	0.1	0.1
	105.0	134.8	6.3	8.4	9.3	9.5	12.3	0.0	0.1	0.1
	107.8	108.8	27.4	32.7	34.7	53.3	22.1	0.1	0.1	0.1
	99.6	90.7	34.6	47.7	53.2	19.5	22.2	0.1	0.1	0.1
	101.9	95.9	20.3	25.0	27.5	20.0	46.2	0.1	0.1	0.1
	97.3	102.3	16.1	11.0	15.3	4.9	16.7	0.0	0.0	0.1
	103.4	84.6	22.8	66.0	31.6	18.0	25.3	0.1	11.2	0.5
	106.1	83.7	40.1	43.2	45.8	19.6	124.8	0.1	0.2	0.1
	94.7	89.2	13.3	15.1	16.2	3.6	81.7	0.1	0.1	0.1
	108.1	101.9	32.4	62.7	36.9	14.9	130.6	0.1	1.1	0.1
	94.2	111.1	42.6	35.0	31.5	10.0	46.4	0.1	0.1	0.1
	90.7	113.4	30.7	32.1	37.9	18.5	28.8	0.1	0.3	0.3
	108.3	139.5	30.4	28.1	27.9	24.6	45.1	0.0	0.1	0.1
	107.3	83.2	9.7	10.2	12.9	15.3	31.5	0.1	0.1	0.1
	89.2	100.9	45.2	33.2	39.1	48.1	75.5	0.1	0.1	0.1
	92.7	98.5	28.3	18.9	16.4	7.8	48.9	0.0	0.0	0.1
	105.3	138.1	10.7	47.6	41.2	7.3	19.6	0.0	3.0	2.1
	106.7	107.8	7.3	24.7	20.4	6.6	9.9	0.0	3.1	1.6
	103.2	109.6	7.6	6.7	8.9	3.0	27.3	0.0	0.0	0.0
	107.2	118.5	15.7	51.1	53.6	10.9	224.0	0.1	3.0	0.8
	103.6	135.5	38.5	40.9	64.1	45.5	72.3	0.1	0.8	1.1
IFG	116.7	126.2	28	53	34	11.5	38.0	0.1	3.1	0.7
	121.7	87.7	22	31	27	11.5	21.5	0.1	0.2	0.1
	121.4	123.6	13	14	15	17.3	14.0	0.1	0.1	0.1
	112.4	108.0	28	76	149	14.1	42.8	0.1	8.2	16.2
	122.4	85.8	17	169	46	9.2	62.2	0.0	30.4	2.0
	116.5	131.1	20	71	74	8.7	38.2	0.1	8.2	4.7
	117.6	111.8	46	54	45	24.7	29.3	0.1	0.2	0.1
	118.4	118.5	21	43	73	20.9	118.5	0.1	0.8	2.6
	111.8	135.2	22	80	140	17.3	62.2	0.1	10.4	19.1
IG	116.5	142.6	59	58	64	38.1	61.9	0.1	0.8	1.3
	107.5	160.1	20	24	29	14.0	67.6	0.1	0.1	0.3
	101.8	142.9	25	54	42	28.1	67.8	0.0	1.0	0.2
	101.6	140.7	8	15	12	3.5	33.9	0.0	0.1	0.1
	88.9	142.5	10	20	33	7.1	17.3	0.1	0.8	1.3
	107.1	178.0	20	20	26	9.7	24.5	0.1	0.1	0.2
	108.3	141.6	16	20	38	11.7	61.4	0.0	0.3	1.1
	125.6	159.4	18	76	115	18.8	27.7	0.1	8.3	18.0
	106.0	159.8	46	66	180	52.2	95.1	0.1	0.2	6.2
	111.0	142.5	11	22	61	6.3	40.5	0.1	0.9	2.8
	121.1	147.0	13	23	17	8.9	12.6	0.1	1.1	0.4
	123.8	152.4	71	179	249	117.5	110.0	0.1	5.7	9.5
	122.6	148.0	31	87	184	19.7	478.9	0.1	3.8	10.2
	123.0	193.8	17	58	156	5.4	160.6	0.1	10.1	25.9
	121.8	146.6	18	94	73	10.3	47.4	0.1	9.0	4.2
糖尿病型	133.4	241.7	20	30	69	7.7	83.4	0.1	1.2	10.0
	133.8	197.7	15	115	313	7.5	360.7	0.1	19.3	71.4
	133.5	142.4	21	53	62	18.4	43.2	0.1	5.6	4.2
	126.7	145.4	20	115	213	23.5	212.9	0.1	25.5	41.1
	120.2	220.1	36	125	186	24.9	235.1	0.1	25.5	27.7
	133.0	260.5	19	82	231	14.9	139.9	0.1	3.3	32.7
	123.7	209.6	35	98	144	27.4	73.9	0.1	3.1	3.9
	131.6	192.7	56	284	233	27.1	68.2	0.1	27.1	18.1
	129.0	184.6	19	136	91	25.2	106.3	0.1	25.7	5.9
	166.7	242.9	35	101	205	42.0	74.9	0.1	9.9	22.6
	123.0	265.8	22	25	123	7.5	94.3	0.1	0.4	17.6
	141.8	199.3	41	80	208	27.2	72.7	0.1	1.0	9.0
	129.3	226.8	39	107	198	25.8	92.1	0.1	9.4	33.3
	127.0	116.0	19	34	78	8.6	31.8	0.1	2.8	7.1

[0126]
[Effect of the Invention] A boundary mold, and IGT and IFG on a boundary with whether according to this invention, it is the normal type with which the candidate has not done the illness of the diabetes mellitus using samples, such as a blood serum, plasma, and urine, and diabetes mellitus Or the inspection approach of the diabetes mellitus judged with simple and sufficient repeatability, especially a diabetes-mellitus reserve is offered [whether it corresponds to diabetes-mellitus reserves, such as insulin resistance, and].